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A STUDY OF THE GERMINATION POTENTIAL IN *MYRIOPHYLLUM SPICATUM* L. SEEDS

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ABSTRACT

Vegetative fragmentation is considered the main mode of propagation of the nuisance aquatic plant, Myriophyllum spicatum L. However, the results presented here demonstrate that the germination potential of Myriophyllum spicatum seeds is high enough that seed germination may be an important mechanism of introduction and re-infestation. Seeds transported by waterfowl may result in the colonization of new habitats. Also, seed germination may result in re-infestation in locations where plants have been eradicated.

Several factors affecting seed germination were investigated. In laboratory studies, seeds received cold storage treatment in 4°C water and in ice for a range of time periods to simulate overwintering conditions. After the various treatments, seeds were placed under Gro-Lux lights at 22°C. The percentage of seeds germinating increased as the duration of cold storage treatment was increased from 4 to 12 weeks, reaching a maximum of approximately 80%. A similar percentage of seeds germinated after treatment with various combinations of cold and freezing as seeds simply treated with cold for the same total period of time. Almost no germination occurred in the dark at 18-20° C.

After seed formation, intact fruits were removed from fruiting spikes at various dates to assess percent germination of seeds not subject to cold treatment. Less than 10% of seeds germinated when removed less than 3 weeks after formation. Larger numbers of seeds germinated when removed 3-4 weeks after formation and the seeds often continued to ripen when stored in warm (20-22°C) water. The highest germination rate of seeds which ripened in the laboratory without cold treatment was 56%.

Large numbers of seeds (70-80%) held over winter in cool, dark conditions in the laboratory germinated when placed in the field in warm, light conditions in glass vials in early summer. Seeds overwintered in cool, dark conditions in situ, then placed on sandy lake bottom at a depth of 1.5 m, showed initial germination of 24% but were overgrown by Chara sp. Although germination potential of overwintered seeds is high, survival of seedlings depends on calm water and low competition with aquatic macrophytes and algae.

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1. INTRODUCTION

During 1977-1979, the Ministry of Environment's Aquatic Plant Management Program attempted to confine rapidly expanding nuisance populations of the aquatic macrophyte Myriophyllum spicatum L. through intensive control and quarantine programs. The possibility of M. spictum propagating from seeds was investigated because transportation by waterfowl with subsequent infestation of uncolonized lakes would jeopardize the program objective of control and confinement. Furthermore, weed management activities such as herbicide treatments, lake drawdown and dredging would be less effective if viable seeds remained to reinfest treated areas.

Seed germination of Myriophyllum spicatum L. (common name Eurasian water milfoil) has been reported in laboratory studies elsewhere (Guppy, 1897; Patten 1955, 1956; Lekic, 1972; Stanley and Goode, 1972; Davis et al., 1973; Collier and Jones, 1980) but with little consistency of experimental design in regard to uniform storage conditions, duration of seed storage, treatment techniques and germination conditions. In some laboratory studies (Patten, 1956; Collier and Jones, 1980), high percent germination was achieved when seeds were subjected to specific conditions known to occur in nature. For example, Patten (1956) reported 85% germination of seeds after treatment by freezing in water, which he stated was a typical condition that M. spicatum seeds naturally encounter in Lake Musconetong, New Jersey. In another study (Collier and Jones, 1980) the importance of red light to produce high percent germination in M. spicatum seeds was demonstrated, a condition confirmed as being important in nature for breaking dormancy in many other seeds (Toole, 1973).

This paper documents a series of experiments initiated in 1978 to determine the effect of cold storage treatment, freezing, light, location on seed spike, plus collection and handling methods on the germination of M. spicatum seeds. These treatments were selected to test experimental methodology and simulate certain overwintering conditions which M. spicatum seeds might receive in British Columbia.

In addition, experiments were conducted to determine M. spicatum seed germination and seedling survival in situ and to determine the potential for spread of this nuisance aquatic plant through sexual reproduction, rather than through vegetative propagation.

2. MATERIALS AND METHODS

2.1 GERMINATION PROCEDURES IN THE LABORATORY

Stems with fruiting spikes of M. spicatum were collected from several locations in Skaha and Okanagan Lakes in October, 1978 and August, 1979. Stems were hand-picked by SCUBA divers from rooted plants in approximately 2 m depth or picked off the beach from recently washed-up plant material. Collected plant material was kept cool and wet while being shipped in coolers to the laboratory. Here fruiting spikes were hand-separated from stem material. Fruits and individual seeds were separated from fruiting spikes by gently rubbing the fruiting spikes while held under water. Individual seeds in nutlets (Figure 1) were then separated from the fruits and other stem material by passing them through a 20-mesh sieve using as little abrasive action as possible. During sorting and separation the exposure of seed spikes to warm air was never long enough to allow complete drying. After separation, seeds were subject to various periods of cold or warm, dark storage treatment in water.

After laboratory treatments, the seeds were stimulated to germinate by exposure to light and warm temperature. Seeds were placed in clear plastic 200 ml cups filled with 20 grams of heat-treated (24 hr. at 125°C) Skaha Lake littoral sediment (90.7% sand, 7.5% silt, 1.8% clay) and 150 ml distilled water. For each type of seed treatment, percent germination was usually observed in 10 cups, each containing 20 seeds placed on the sediment surface. The cups were placed on a laboratory bench at room temperature (19-22°C) under four GTE Sylvania Gro-Lux fluorescent lights. The number of germinated seeds was recorded weekly for up to 14 weeks.

Gro-Lux fluorescent lights were suspended approximately 0.25 m above the laboratory bench on which seed germination experiments were run. The 1.2 m tubes were maintained on a 14 hour light-10 hour dark cycle. Natural daylight was not available. Gro-lux lights were chosen as a suitable source

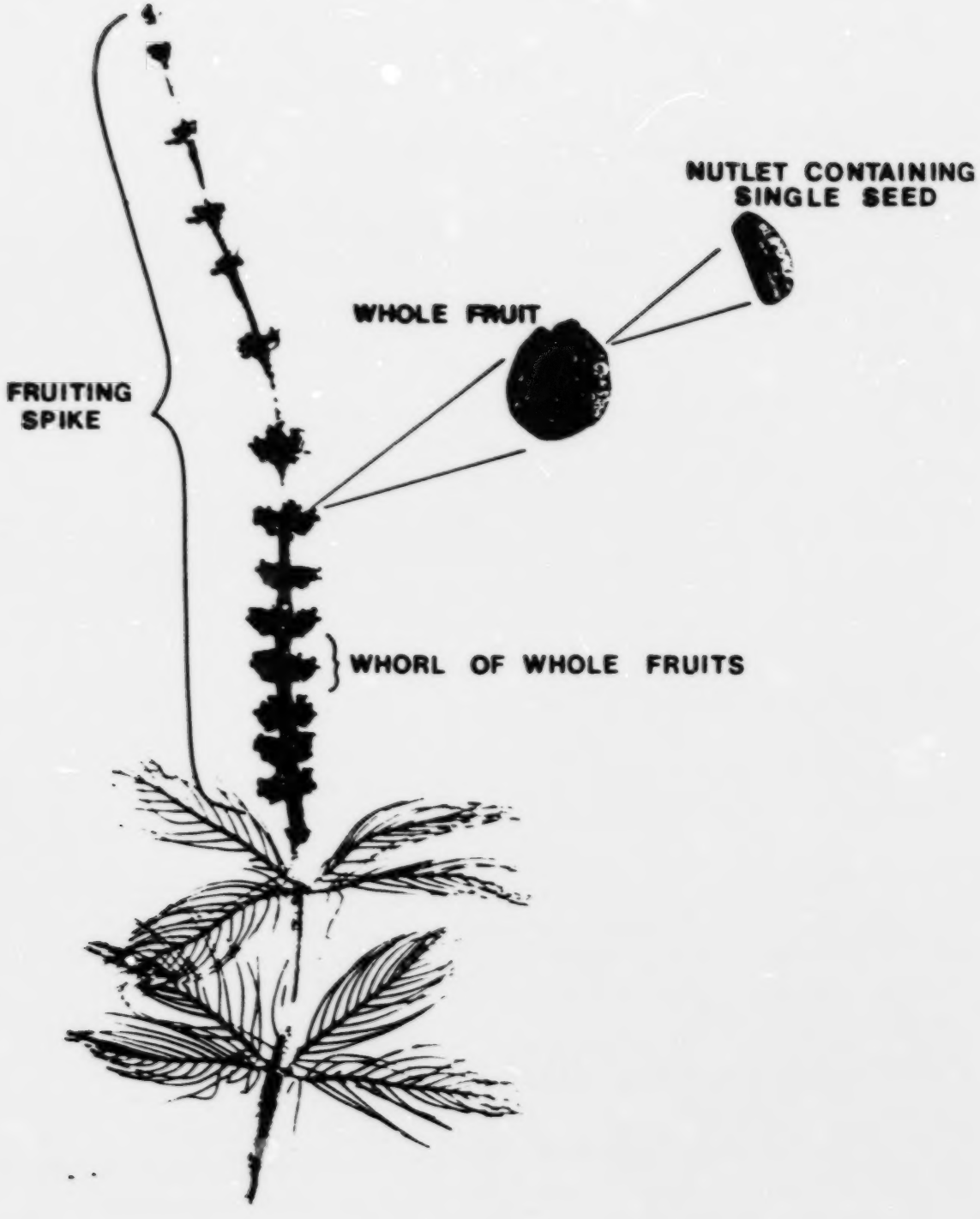

Figure 1. Fruiting Spike of Myriophyllum spicatum L. (x2)

of artificial light because they emit light energy primarily in the red (40%) and blue (27%) regions of the visible spectrum (630-700 nm and 430-490 nm respectively). Red and blue light are considered essential for photosynthesis and seed germination (Anon. N.P.D.; Toole, 1973).

Light intensities of red and blue light were measured at various distances from Gro-Lux lights using a Kahlsico submarine photometer (model number 268 WA 310). Red and blue colour filters measured light in the respective spectral ranges of 620-680 nm and 420-480 nm. At a distance of 0.25 m from the lights, the intensity of red light was approximately 0.65 g cal/cm^2/hr and 0.25 g cal/cm^2/hr for blue light (see Figures 2 and 3). In addition, direct sunlight was measured for comparative purposes at 1500 hours in mid-March at 48°26' north latitude. Red light was measured as 1.58 g cal/cm^2/hr and blue light was measured as 1.8 g cal/cm^2/hr. At a distance of 0.25 m from Gro-Lux lights, seed germination cups received approximately 40% and 14% of these natural daylight values of red and blue light respectively.

2.2 TREATMENTS OF SEEDS BEFORE GERMINATION IN THE LABORATORY

2.2.1 SEEDS FROM TWO COLLECTION SITES

M. spicatum stems with fruiting spikes were collected from two locations near Kaleden in Skaha Lake. Plant material collected from one location was representative of a specific stand of M. spicatum, growing offshore at a depth of 2 m. At the time of the collection (October 3, 1978), the mature fruiting spikes were submerged. Plant stems collected here were kept cool and wet before transfer to the laboratory refrigerator for cold storage treatment at 4°C in the dark. Plant material collected from the other location was representative of partially air-dried fruiting spikes which had washed up on shore. In most cases, the spikes were still attached to whole plant tops which were gathered in bunches, placed in coolers and doused with water to prevent complete drying (October 12, 1978).

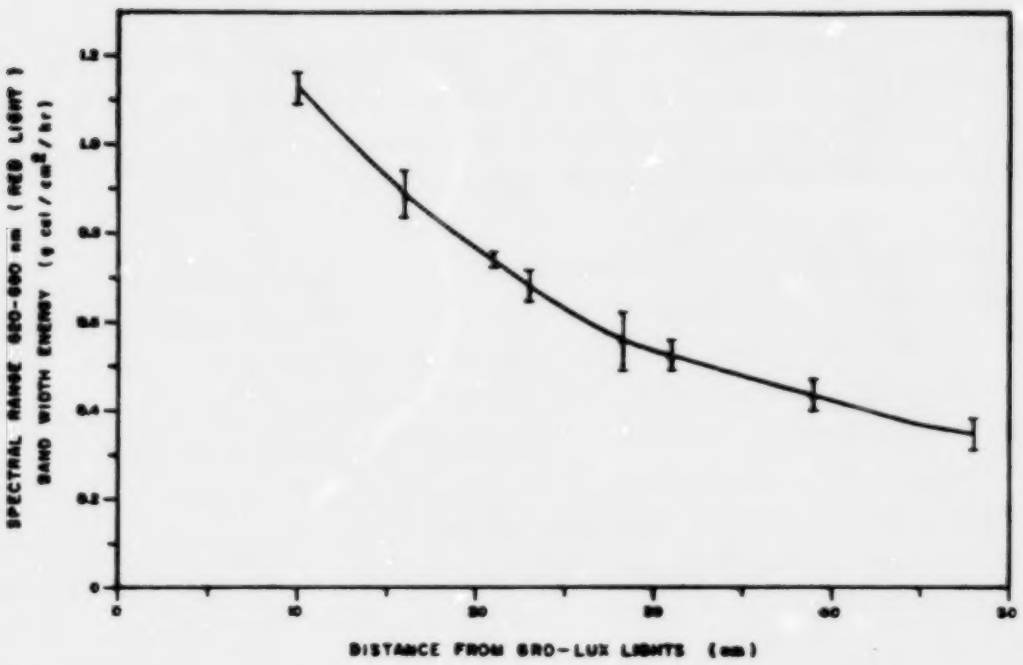

Figure 2. Mean Spectral Energy of Red Light Emitted from Four GTE Sylvania Gro-Lux Lights with 95% Confidence Limits

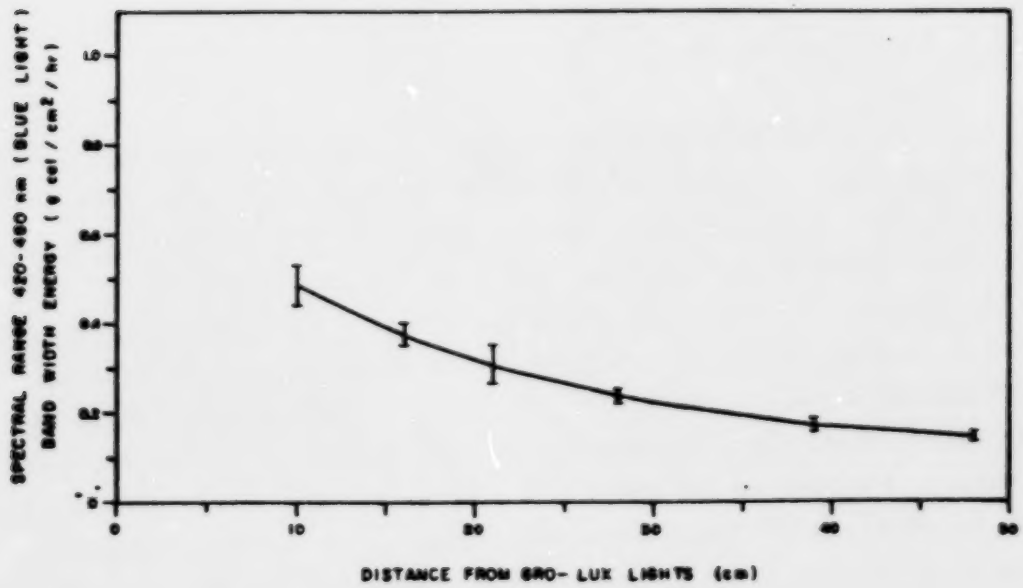

Figure 3. Mean Spectral Energy of Blue Light Emitted from Four GTE Sylvania Gro-Lux Lights with 95% Confidence Limits

Percent germination of seeds from these two locations (offshore and shore-washed) were compared following different periods of cold, dark storage treatment at 4° C.

2.2.2 SEEDS SUBJECT TO COLD, DARK STORAGE TREATMENT

Percent germination of seeds from both shore-washed and offshore locations near Kaleden in Skaha Lake were compared after 4,6,8,12,20 and 28 weeks of cold, dark storage treatment at 4°C.

2.2.3 SEEDS SUBJECT TO WARM, DARK STORAGE TREATMENT

After 4 and 6 weeks of cold, dark storage treatment, a group of offshore seeds were held in the dark at room temperatures of 18-20°C for 8 and 22 weeks. The subsequent failure of these seeds to germinate under warm, dark conditions indicated that further assessment of germination in the dark was unnecessary. These seeds were then exposed to light and germination observed again for each treatment condition.

2.2.4 SEEDS FROM DIFFERENT LOCATIONS ON FRUITING SPIKES

Another seed germination experiment was initiated to study the effect of seed location along the fruiting spike. Seeds collected from offshore plants near Kaleden were placed into separate petri dishes which represented each of the eight seed whorls (proximal whorls were used if more than eight whorls were present) along the length of the fruiting spikes (Figure 1). On many fruiting spikes, even the eighth, distal whorl did not have fully developed seeds. All seeds were treated with six weeks of wet, dark, cold storage at 4°C and percent germination was then compared for each whorl location.

2.2.5 COMPARISON OF SINGLE SEEDS WITH WHOLE FRUITS

In another experiment, the germination of single, hand-separated seeds was compared with the germination of seeds fused together in whole fruits. Seeds and fruits were subject to 12 weeks of cold, dark storage treatment. Five separate fruit whorls were placed on sediment in each of 10 cups containing distilled water. Each fruit whorl consisted of 2-5 fruits with each fruit consisting of 2-4 seeds. The total number of seeds in the 50 fruit whorls tallied 573; single seeds tallied 200 (10 cups each containing 20 seeds). Both groups of fruit whorls and single seeds were collected from the offshore site at Kaleden, Skaha Lake.

2.2.6 SEEDS SUBJECT TO FREEZING TREATMENT

In order to assess the effects of various combinations of cold and freezing, three groups of offshore seeds from Skaha Lake were treated with 8,12 and 20 weeks of cold storage treatment at 4°C and frozen (-5°C) in distilled water for 13, 4 and 1 week periods respectively . After freezing, the seeds were returned to cold storage in 4°C water until a total of 28 weeks of treatment had passed. Percent germination was determined for each of the three treatment groups.

2.2.7 SEEDS OF DIFFERENT MATURITY

In 1979, fruiting spikes which appeared to have ripened *in situ* for various time periods were collected from two sites, one near Whiteman Creek outlet into Okanagan Lake and the other near Kaleden on Skaha Lake. Seed germination in these various collections were compared, in some cases after being subject to various periods of storage treatment in the lab, in warm water and under low light intensities, to allow additional ripening as follows:

i) Whiteman Creek seeds collected August 1, no storage treatment

Seeds collected on August 1, 1979 were green, hard and tightly bound in whole fruits. Their condition suggested that they were young seeds which had most likely formed in late July. One hundred whole fruits (328 seeds) were removed from fruiting spikes and percent germination recorded for seeds remaining bound in these fruits.

ii) Whiteman Creek seeds collected August 23, stored for five days

Although these seeds collected August 23 had an additional three weeks to ripen in situ, they were still bound together as intact fruits. After collection, fruiting spikes were subject to five days of warm (20°C) lake water, dark storage treatment. One hundred whole fruits (350 seeds) were removed from fruiting spikes and percent germination recorded for seeds remaining bound in these fruits.

iii) Whiteman Creek seeds collected August 23, stored for four weeks

After collection, these fruiting spikes were subject to four weeks of warm (20°C) lake water storage treatment in diffuse light. These fruits had darkened and softened to such an extent that when handled, they crumbled into their component seeds. Percent germination was recorded for these separated seeds.

iv) Kaleden seeds collected August 9, stored for four days

These seeds were still green and loosely bound in whole fruits. The relative ease at which they could be separated suggested that they were several weeks older than the Whiteman Creek seeds. They had likely formed in mid-July rather than in late July. These fruits were subject to four days of warm storage treatment in the dark during transit from the field to the laboratory. Percent germination was recorded for separated seeds.

v) Kaleden seeds collected August 9, stored for two weeks

After collection, fruits were divided and individual seeds were stored for two weeks in warm (20°C) tap water under low light. Percent germination was recorded for separated seeds.

vi) Kaleden seeds collected August 9, stored for seven weeks

These seeds were stored for seven weeks in warm (20°C) tap water under low light. Percent germination was recorded for separated seeds.

2.3 SEED GERMINATION IN THE FIELD

2.3.1 *IN SITU* GERMINATION AFTER EIGHT MONTHS COLD STORAGE TREATMENT IN THE LABORATORY

To simulate overwintering followed by gradual spring warming, seeds collected from Skaha Lake in October, 1978 were maintained in dark, wet, cold storage at 4°C in a laboratory fridge for a period of eight months. Seeds were then transferred to a 4.5 L plastic bottle with holes punched to allow water circulation and placed in Vernon Arm, Okanagan Lake for two months of *in situ* storage treatment in the dark (May 25 to July 23, 1979).

Following *in situ* storage treatment, ten 25 ml glass vials were each filled with distilled water and 20 *M. spicatum* seeds. Vials were then capped and placed on the bottom at a depth of 1.5 m in Vernon Arm. After 17 days, no germination was observed and small holes were made in each of the vial lids to allow the exchange of water and gases. Additional observations were made after two weeks.

2.3.2 *IN SITU* GERMINATION IN AUTUMN AND SPRING FOLLOWING *IN SITU* STORAGE TREATMENT

Seeds that were collected from an *M. spicatum* site near Fintry, Okanagan Lake on August 24, 1979 were used in two *in situ* seed germination experiments.

Fruiting spikes were collected and stored underwater for 10 and 15 weeks in Okanagan Lake, Vernon Arm in a 4.5 L plastic bottle. In order to allow circulation of water and gases, holes were punched in the bottle as well as in the dark plastic bag which was placed around it to exclude light.

In order to stimulate germination in each of the following two experiments, 200 seeds were randomly selected and transferred to glass jars with holes in the lids. These jars were placed on the lake bottom at a depth of 1.5 m, exposed to natural sunlight, and protected from wave action.

2.3.2.1 Ten Week *In Situ* Storage Treatment

On November 1, 1979, after 10 weeks of *in situ* storage treatment, the first group of seeds was transferred to the glass jars.

Observations on seed germination were made by a SCUBA diver on November 16 and 19, 1979 and then again on April 29, 1980 and May 5, 1980 at which time the experiment was terminated.

2.3.2.2 Fifteen Week *In Situ* Storage Treatment

On December 7, 1979, after 15 weeks of *in situ* storage treatment, a second group of seeds was transferred to glass jars.

Due to winter ice formation over Vernon Arm, observations on seed germination and water temperature were not made until, April 29, 1980 and finally on May 5, 1980 at which time the experiment was terminated.

2.3.3 *IN SITU* GERMINATION AND SURVIVAL OF SEEDLINGS AFTER *IN SITU* OVERWINTERING OF SEEDS

Additional observations on germination of overwintered seeds were made in the summer of 1980 using seeds collected near Fintry in Okanagan Lake on August 24, 1979. In this experiment, germination of seeds placed on the lake bottom and seedling survival were observed.

After nine months of *in situ* storage in darkness, the seeds were transferred (June 3, 1980) to four clear plexiglass shelters which were placed on the lake bottom at a depth of 1.5 metres. Each four-sided pyramid-shaped shelter covered an open base area of 0.25 m^2. The lake bottom under each shelter was cleared of algae and debris by SCUBA divers and 50 seeds were placed on the sandy bottom. Small holes were drilled in the sides of each shelter to allow circulation of lake water. Observations on seed germination were made biweekly until July 25, 1980, at which time the shelters were removed. Additional observations were made on August 1 and 22 before termination of the experiment due to dominance of the alga *Chara* sp. throughout the area.

2.4 DATA ANALYSIS

Seed germination experiments dealt with the proportions of *Myriophyllum spicatum* seeds that germinated after receiving various treatments. These types of data were not normally distributed and were not amenable to standard analysis of variance. Means, variances and confidence limits were calculated, however, and contingency tables were used to test for differences between the mean proportions of seeds which germinated in each

experiment. Contingency tables test for multiplicative differences between means whereas analysis of variance tests for additive differences and interactions between means (Everitt, 1977).

In order to test for overall independence of variables, three groups of data were combined to form a three-dimensional (three-way) contingency table. The three variables tested were: duration of cold storage treatment (weeks), seed collection location (offshore or shore-washed) and seed germination (proportions of seeds which germinated, or did not germinate). Results were significant using chi-square statistics and implied that there were associations between some, but not necessarily all variables. Further testing was performed to determine which variables were associated.

3. RESULTS AND DISCUSSION

3.1 SEED GERMINATION IN THE LABORATORY

3.1.1 SEEDS FROM TWO COLLECTION SITES

Mean germination rates of seeds collected from an offshore population and from shore-washed plants near Kaleden in Skaha Lake are compared for various cold storage periods in Table 1. All means are presented with 95 percent confidence limits. For both groups of seeds, there was only a small difference in percent germination for cold treatment periods ranging from 4 to 28 weeks. Germination ranged between 62 ± 8.8% and 82.5 ± 7.4%.

The three-way contingency table was used to test the hypothesis of partial independence of the effect of seed collection location with the other two variables, length of cold treatment and percent seed germination. Regardless of the length of cold treatment which seeds received, there was no significant difference between the germination rates of seeds collected in the lake and seeds collected on the shore. The exposure which shore-washed seeds received from varying amounts of warming, drying, handling and sorting were therefore considered to be insignificant.

TABLE 1

MEAN SEED GERMINATION RATES ± 95% CONFIDENCE LIMITS (n=200 SEEDS) FOR VARIOUS COLD STORAGE TREATMENT PERIODS FOR OFFSHORE AND SHORE-WASHED COLLECTIONS

SEED COLLECTION SITE	NO. WEEKS COLD STORAGE					
	4	6	8	12	20	28
Offshore	62 ± 8.8%	65.5±11.6%	72 ± 7.6%	77 ± 8.6%	78 ± 9.7%	72.5±7.0%
Shore-washed	67 ± 6.4%	79 ± 6.3%	82.5±7.2%	82.5±7.4%	81 ± 5.8%	72.5±6.6%

3.1.2 SEEDS SUBJECT TO COLD, DARK STORAGE TREATMENT

Germination rates were relatively high for all periods of cold treatment (Table 1), however, there appeared to be a higher germination rate for 8, 12 and 20 weeks of treatment than for 4, 6 and 28 weeks of treatment. Since there was no significant overall difference between germination rates of offshore and shore-washed seeds, germination data from these groups were combined to make a two-way contingency table to determine if there were differences in germination rates of seeds which received different periods of cold treatment. Results were significant and further testing was performed on the data to determine which groups, if any, showed different germination rates.

Although the 95 percent confidence limits to the mean germination rates show considerable overlap (Figure 4), contingency tables were used to check for more subtle differences between means by examining all germination rates simultaneously. The 8, 12, and 20 week germination rates were combined as one group of data (representing the highest observed germination rates) and tested against each of the other treatments in one contingency table. Using chi-square statistics generated from the contingency table, seed germination rates differed significantly ($\alpha = 0.5$) between the groups. The results implied that each of the 4, 6 and 28 week treatments were significantly different from each other, while in turn, each was significantly different from seeds which received 8, 12 or 20 weeks of cold treatment. It appeared that seed germination potential increased significantly during the first eight weeks of cold treatment in 4°C water reaching a maximum of 80 percent for seeds which received 8-20 weeks of treatment. Seeds which received longer cold treatment showed a slight decline in germination potential.

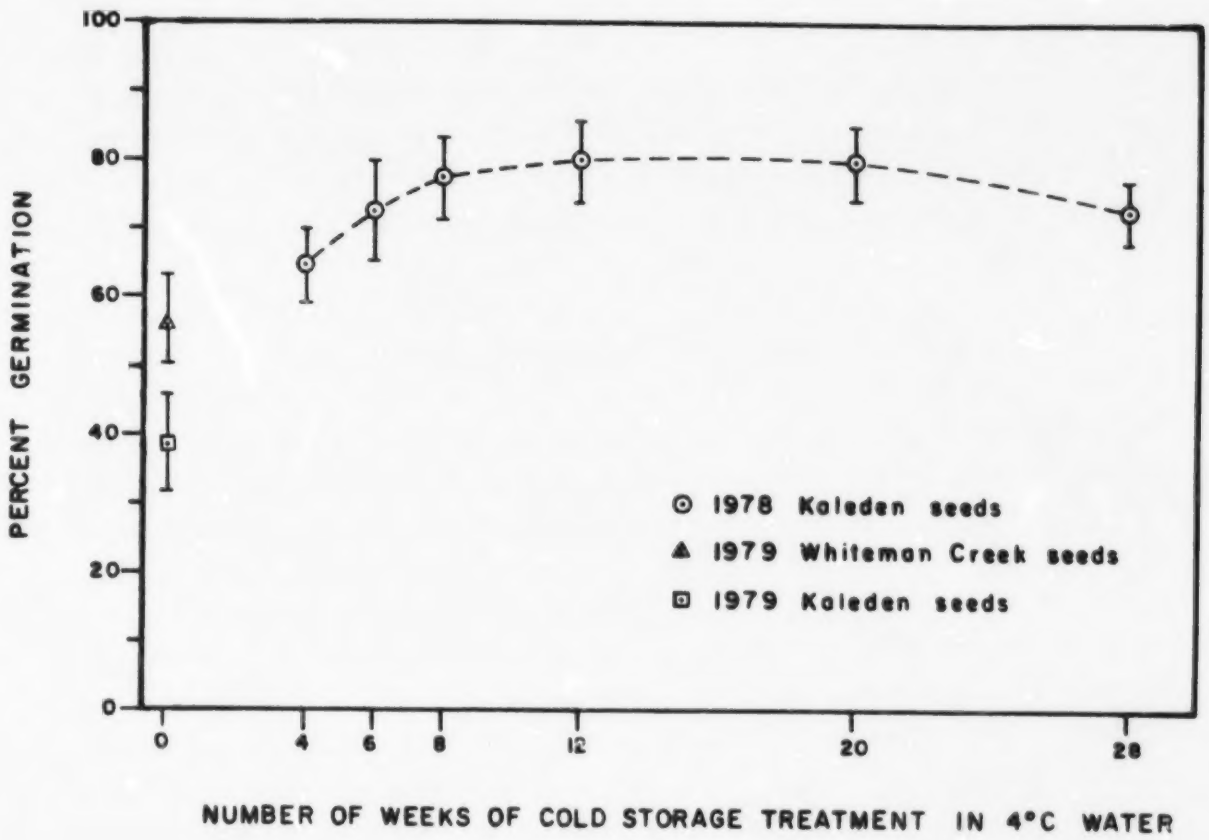

Figure 4. Combined Germination of Offshore and Shore-Collected Myriophyllum spicatum Seeds after Various Periods of Cold Treatment (Mean Germination with 95% Confidence Limits, n = 400 seeds) Compared with Seeds given no Cold Treatment

Seeds collected in 1979 near Kaleden, Skaha Lake and near Whiteman Creek, Okanagan Lake were germinated in the laboratory with no cold storage treatment. Data were compared (Figure 4) with the germination rate of seeds given 4-28 weeks cold storage treatment. Mean germination rates of seeds not subject to cold treatment were 38 ± 6.1% and 57 ± 6.3%, which were significantly lower than germination after 8-20 weeks cold treatment.

3.1.3 SEEDS SUBJECT TO WARM, DARK STORAGE TREAATMENT

Although Guppy (1897) reported that M. spicatum seeds (of unspecified age) germinated in the darkness in water, more recent work (Collier and Jones, 1980) stressed the importance of light for M. spicatum seed germination. In their study, seed germination in the darkness in water was reported to be less than one percent for seeds which had been stored dry for 5 to 137 months.

In this study, one seed (out of 400 which received four and six weeks of cold treatment in 4°C water) germinated in the darkness in 18-20° water. When these seeds were exposed to light following cold treatment and further storage in the darkness, in warm water, for 8 to 22 weeks, there was 27 ± 6.5% and 44 ± 14.4% germination respectively, which was significantly lower than germination of seeds which received only cold treatment for the same length of time (Figure 5). Seeds which received storage treatment in warm water in the dark seemed partly decomposed at the end of the germination experiments, in contrast to the healthy appearance of seeds which received no warm storage treatment. The results suggest that in lakes where seeds receive insufficient light to allow seed germination, cold water will enhance germination potential of overwintering seeds while reducing the effects of decomposition. However, as lake water warms in spring and summer, seeds which are not exposed to light will lose germination potential as seeds begin to decompose.

3.1.4 SEEDS FROM DIFFERENT LOCATIONS ON FRUITING SPIKES

Germination potential did not vary significantly between seeds taken from each whorl along fruiting spikes. Germination ranged from 78 ± 7.1 to 95 ± 6.2%. The results indicate that a systematic bias in the many germination trials in this study could not occur as the result of seeds from a particular portion of the spike predominating in certain collections.

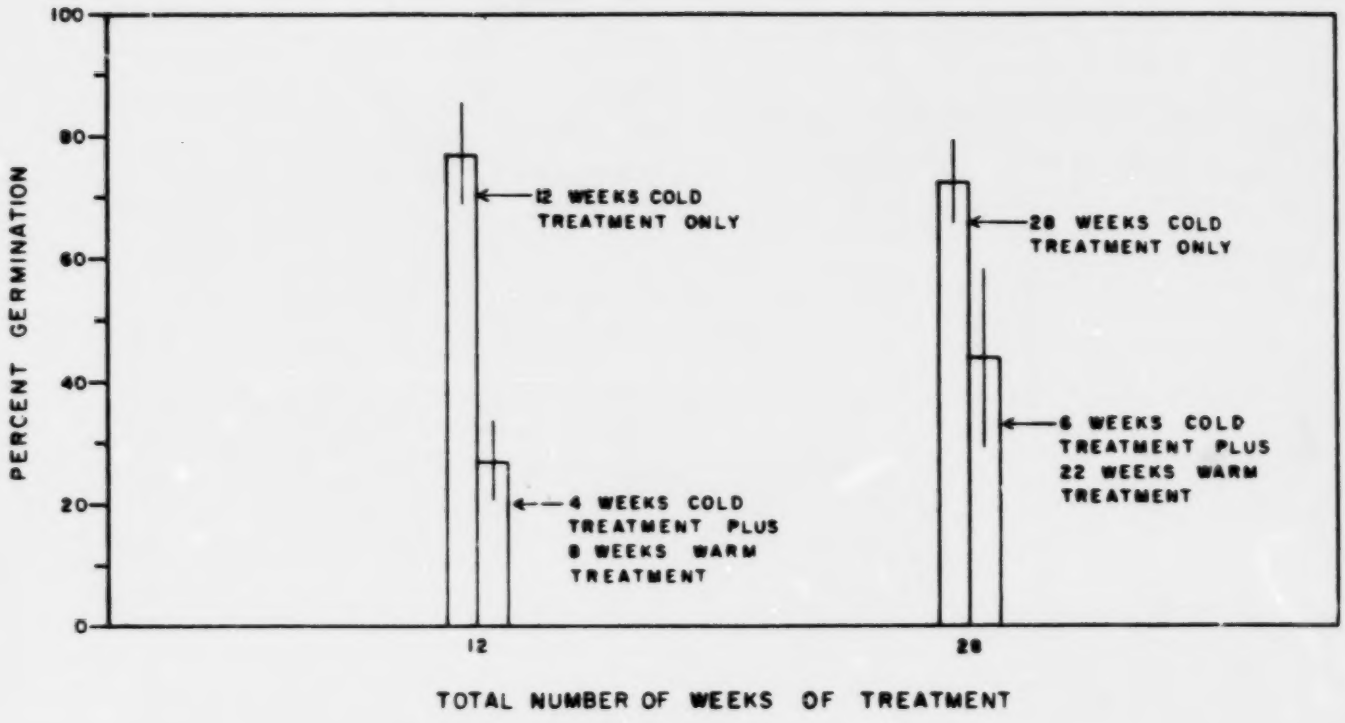

Figure 5. Germination of Seeds Given Cold Storage Treatment in 4°C Water Compared with Seeds Given Equivalent Periods of Storage in 4°C and 20°C Water. (Mean Germination with 95% Confidence Limits, n = 200 Seeds)

3.1.5 COMPAARISON OF SINGLE SEEDS WITH WHOLE FRUITS

After 12 weeks of cold treatment in 4°C water, followed by exposure to light, single seeds began to germinate more quickly than seeds in whole fruits. These results are similar to the more rapid initial germination rates of single seeds than whole fruits, reported by Collier and Jones (1980). However, after 6 weeks exposure to light, the cumulative percent germination of whole fruits had increased substantially to 83 percent. Final percent germination was not significantly different between single seeds and seeds in whole fruits (Figure 6).

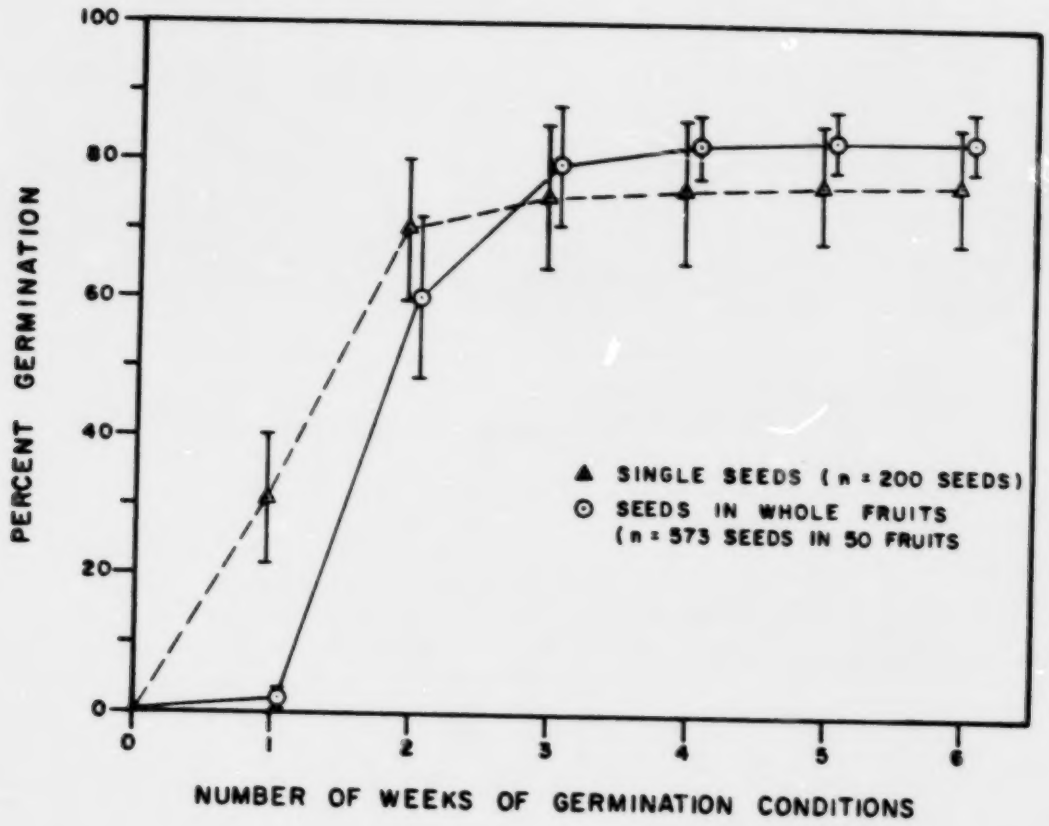

Figure 6. Mean Germination of Single Seeds (±95% C.L.) Compared with Seeds in Whole Fruits after 12 weeks of Cold Storage Treatment in 4°C Water

3.1.6 SEEDS SUBJECT TO FREEZING TREATMENT

Since many littoral zones infested with M. spicatum freeze for varying periods during winter months in British Columbia, seeds were subjected to 1,4 and 13 weeks of freezing during part of their total 28 week cold treatment period. All three seed groups subject to freezing and cold treatments showed high percent germination (73±7.4% to 80±8.6%) which was similar for the seeds which received the 28 week period of cold treatment only (72±7.0%) (Figure 7). There were no significant differences in percent germination between the three groups subjected to freezing. These results indicate that combined freezing (-5°C) and cold (4°C) treatments are equivalent to cold treatment only in enhancing seed germination.

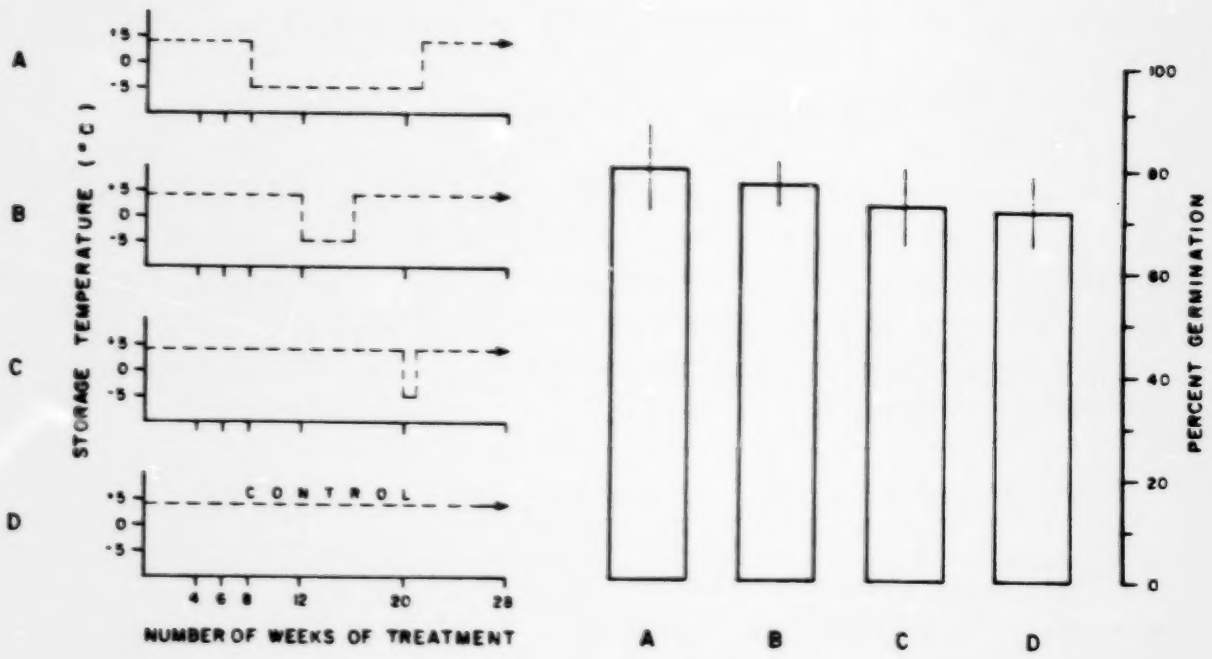

Figure 7. Germination of Seeds after Various Periods of Freezing (-5°C) and Cold Water (4°C) Treatment Compared with Control Seeds given Cold Water Treatment only. (Mean Percent Germination with 95% Confidence Limits, n=200 Seeds)

3.1.7 SEEDS OF DIFFERENT MATURITY

Results of experiments testing percent germination of seeds of varying maturity which were treated in the laboratory under low light, warm water storage conditions (18-20°C) are illustrated in Figures 8 and 9.

i) Whiteman Creek seeds, collected August 1, no storage treatment

Seeds from Whiteman Creek, Okanagan Lake collected on August 1, 1979 were considered to be immature because the nutlets containing seeds were green, hard and tightly fused in whole fruits. The immaturity of these seeds was confirmed by their low germination (8 ± 5.2%) when they were exposed to full light in the laboratory with no prior storage period.

ii) Whiteman Creek seeds, collected August 23, stored for five days

These seeds also appeared immature, although they had remained *in situ* in fruits attached to plant stems for three weeks longer than the first collection and were stored in the laboratory for an additional 5 days. Whole fruits were removed from stems prior to full exposure to light. Mean final percent germination was low and (18.3 ± 8.6%) not statistically different from the previous collection.

iii) Whiteman Creek seeds, collected August 23, stored for four weeks

These seeds appeared to have matured considerably after four weeks of warm water storage treatment under diffuse light in the laboratory while still remaining in fruits attached to plant stems. Germination was significantly higher (56.5 ± 6.7%) than in the previous two groups of seeds (Figure 8).

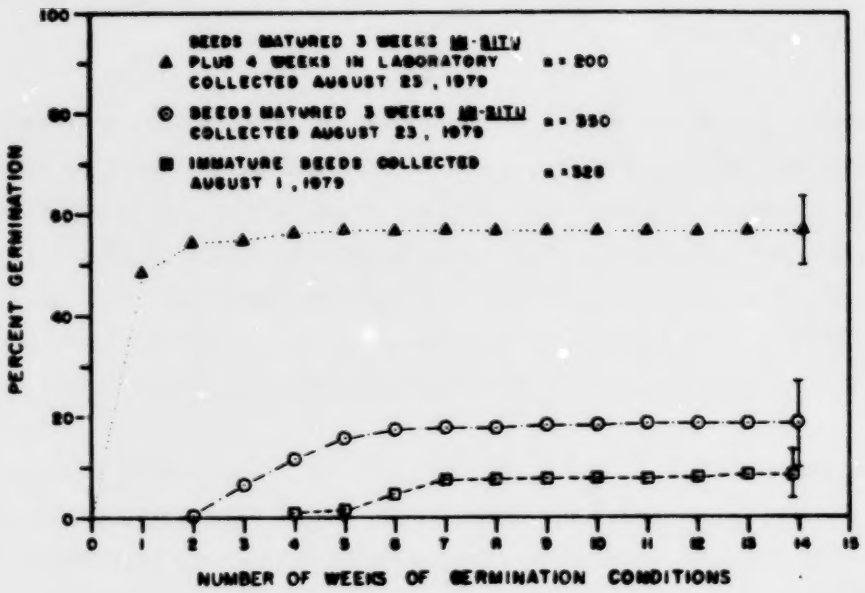

Figure 8. Germination of Immature Seeds at 22°C, Two Days after Field Collection (Whiteman Creek, Aug. 1, 1979) Compared with Seeds Given Additional Time to Mature In Situ and in the Laboratory. (Mean Final Percent Germination with 95% Confidence Limits)

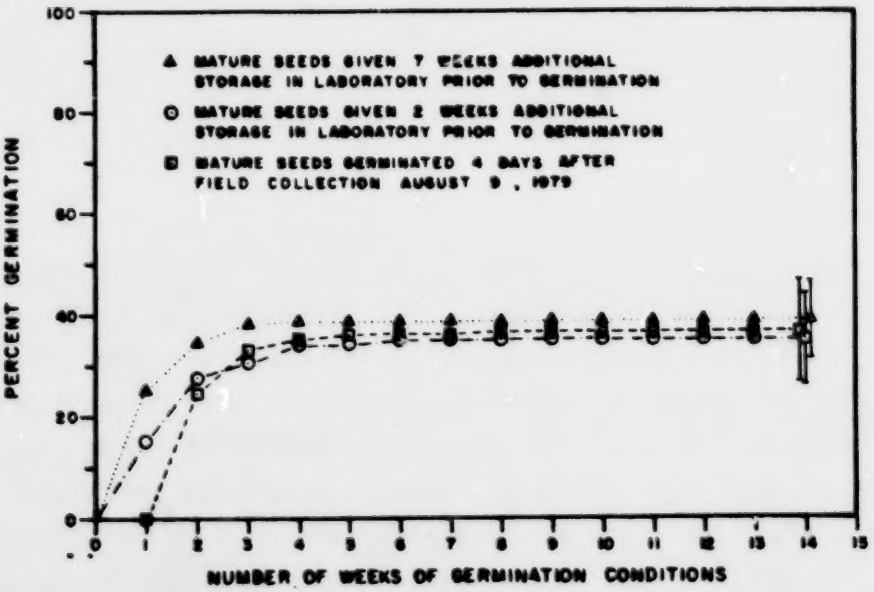
MATURE SEEDS GIVEN 7 WEEKS ADDITIONAL
STORAGE IN LABORATORY PRIOR TO GERMINATION
MATURE SEEDS GIVEN 2 WEEKS ADDITIONAL
STORAGE IN LABORATORY PRIOR TO GERMINATION
MATURE SEEDS GERMINATED 4 DAYS AFTER
FIELD COLLECTION AUGUST 9, 1979
PERCENT GERMINATION
NUMBER OF WEEKS OF GERMINATION CONDITIONS

Figure 9. Germination of Mature Seeds at 22°C, Four Days After Field Collection (Kaleden, Aug. 9, 1979) Compared with Mature Seeds Given Additional Warm, Dark, Wet Storage Treatment in Laboratory Prior to Germination. (Mean Final Percent Germination with 95% Confidence Limits, n=200 Seeds)

iv) Kaleden seeds, collected August 9, stored for four days, two weeks and seven weeks.

These seeds, collected August 9, appeared to be more mature than those from Whiteman Creek because the fruits were easily broken down into separate seeds, four days after collection. A similar percentage of seeds from all three storage periods germinated (approximately 37%). The results (illustrated in Figure 9) suggest that the Kaleden seeds had reached a high level of maturity *in situ* and that only a slight additional period was required to bring these seeds to their maximum germination potential.

The maximum percent germination of Kaleden seeds (37%) was significantly lower than Whiteman Creek seeds (56%) (see Figures 8 and 9). The lower germination of Kaleden seeds may be a result of environmental or genetic factors which affected seed development in different ways. Similarily, different conditions within storage containers may have affected further seed development in the laboratory. The removal of Kaleden seeds from fruiting spikes prior to storage treatments may have also limited further seed maturation.

3.2 SEED GERMINATION IN THE FIELD

3.2.1 *IN SITU* GERMINATION AFTER EIGHT MONTHS COLD STORAGE TREATMENT IN THE LABORATORY

Mature *Myriophyllum spicatum* seeds which received simulated overwintering conditions (wet, dark, cold storage in a laboratory refrigerator) followed by exposure to natural lake-warming in the spring (in the dark, *in situ*) showed an estimated germination rate of 70-80 percent after being exposed to daylight in mid-summer at a depth of 1.5 m. The warm water (20-23°C), day length, light intensity and protected environment offered by the glass vials are all likely factors which allowed successful germination *in situ*. The high percent germination of these seeds demonstrated their ability to ripen in late autumn, overwinter and germinate successfully in the spring *in situ*.

3.2.2 IN SITU GERMINATION IN AUTUMN AND SPRING FOLLOWING IN SITU TREATMENT

3.2.2.1 In situ Germination After Ten Weeks of In situ Storage

Seeds which were collected in late summer and stored for 10 weeks until November in the dark in situ showed significant germination when exposed to daylight in November (12 percent germination by mid-November; 26 percent germination by late November). Water temperatures decreased from 13 to 7°C during the month but were obviously adequate enough to allow some germination. Patten (1956) suggests that there is a temperature range (probably 10-20°C) which prevents germination from proceeding readily. Below this temperature range, germination would be inhibited and ripening would be enhanced. Under natural conditions, as water temperatures rise in the spring, overwintered seeds would germinate under more favourable, warmer conditions. Seeds in this experiment overwintered in situ under natural daylight for five months, but were never exposed to temperatures warm enough to allow germination to proceed readily. The maximum germination potential of these seeds was never recognized because they were removed from the in situ experimental site the following spring (May 5, 1980) when maximum water temperatures (13°C) were still well below optimum. Thirty-four percent of seeds had germinated when the experiment was terminated.

3.2.2.2 In situ Germination After Fifteen Weeks of In Situ Storage

Seeds exposed to light in December after 15 weeks of in situ dark storage treatment showed a final in situ germination of 48 percent the following spring (May 5, 1980). The final number of germinated seeds was higher than for seeds which received only 10 weeks of dark storage treatment in situ. The additional month of storage in lake water which cooled from 13 to 7°C appears important. When these seeds were exposed to daylight on December 3, 1979, estimated water temperatures of 6.5°C were likely too low to allow any germination until the spring. Ice formation on the lake prevented observations from being made during the winter months.

Although the experiment was terminated, like the previous experiment, before water temperatures became greater than 13°C, the in situ germination of these overwintered seeds demonstrated the ability of M. spicatum seeds to ripen in the late fall and winter (in the dark or under low light) and germinate successfully in the spring under more favourable conditions.

3.2.3. GERMINATION AND SURVIVAL OF SEEDLINGS AFTER IN SITU OVERWINTERING

Seeds which were collected in late summer and overwintered in the dark in situ began to germinate rapidly when placed on natural lake bottom in early June, the following year. By June 16, 24 percent of seeds laid down under plexiglass shelters had germinated. However, the alga Chara sp. also showed rapid growth in the shelters and by mid-June appeared to be in direct competition with the M. spicatum seedlings for space and light. Although several M. spicatum seedlings developed to a height of 15 cm by July 4, Chara sp. began to dominate the shelters and no further growth of M. spicatum was observed. By July 15, Chara sp. reached a height of 20 cm. Observations on the abundance of M. spicatum became increasingly difficult and when the shelters were removed by SCUBA divers on July 25, no M. spicatum seedlings were apparent. Although several small M. spicatum plants were observed in the area in late August, their origin (fragment growth or germination from seed) was uncertain.

Competition for light and space by aquatic plants such as Chara appeared to be a major factor limiting the survival of M. spicatum seedlings in situ. In contrast, M. spicatum seedlings have been observed to develop into mature plants in the laboratory under controlled environmental conditions (Stanley and Goode, 1972; this study). However, under natural conditions, seedlings have never been positively identified in the field by SCUBA divers over the many years (1977-1980) of observations relating to this and other studies in the Okanagan Valley. In contrast, colonization from the rooting of small fragments was frequently observed.

4. CONCLUSIONS

The results of the germination experiments demonstrate that the germination potential of M. spicatum seeds is highly variable, being affected by cold treatment, ripening conditions and other environmental or genetic factors. Germination potential is induced by cold treatment, indicating that winter conditions (in temperate climates) are necessary for maximizing seed germination potential.

The inability of seeds to germinate in the absence of light demonstrates that germination in the field will be greatly limited by depth and substrate type. Seeds deposited in water not subject to the effects of wave-washing are likely to be buried by sedimentation processes. Seeds deposited in shallower, wave-washed areas are more likely to be stimulated to germinate at various times as they are exposed to light and suitable water temperatures. However, the failure of seedlings to vigorously root and grow limits their potential for successful establishment in shallow, wave-washed areas.

Under suitable summer conditions, some seeds may germinate shortly after ripening, while others remain dormant. In temperate climates, however, germination in the immediate growing season is considered to be disadvantageous as a survival strategy because seedling survival in late summer and autumn would be expected to be limited by declining water temperatures, reduced light availability and increased competition from periphyton growth.

Weed management techniques that disturb bottom sediments, such as tillage and rotavation, may stimulate seed germination when buried seeds are exposed to light. This stimulation is not, however, considered to be a significant mechanism for recolonization because such treatment methods leave enough vegetative material behind to result in far greater regrowth than is believed would occur from seedling growth.

Seeds ingested and transported by waterfowl may result in the introduction of M. spicatum to new habitats. Given a sequence of events which provides sufficient time for ripening and cold treatment, seeds transported to new habitats may germinate when exposed to favourable light and temperature conditions. For a seedling to mature into a plant capable of vegetative reproduction, the seed must be deposited at suitable depth and encounter adequate substrate, water temperature, light availability, water quality and protection from excessive wave exposure. Upon germination, the seedling must then survive competition from algae and other aquatic plants. The probability of this sequence of events occurring is extremely small, but given the millions of viable seeds produced by plant populations every year, the introduction of M. spicatum to new habitats by waterfowl transport is inevitable.

5. SUMMARY

1. Cold dark storage treatment in water enhanced seed germination potential. The percent germination of seeds treated 8-20 weeks (about 80 percent) was somewhat higher than seeds treated for 4, 6 and 28 weeks (about 70 percent) and for seeds not exposed to any cold treatment (30-60 percent).

2. No significant difference was detected in the percent germination of seeds collected from rooted M. spicatum stems compared with seeds from shore-washed fruiting spikes.

3. There was almost no germination of seeds held in the dark at 18-20°C.

4. Continued warm dark treatment of cold treated seeds resulted in a reduction in germination potential.

5. Germination potential did not vary significantly for seeds taken from different whorls along the fruiting spike.

6. There was no significant difference between the percent germination of single seeds and of seeds left in whole fruits.

7. There was no significant difference in percent germination of seeds frozen for various time periods and seeds stored in cold water without freezing.

8. Seed retention on the fruiting spike for 1-2 months after initial formation appeared to be an important requirement for seed ripening and germination.

9. Seeds which overwintered in the laboratory under cool, dark conditions followed by gradual warming in the darkness *in situ* the following spring showed an estimated 70-80 percent germination when exposed to natural daylight and warmer water.

10. Seeds exposed to daylight in November and December and overwintered *in situ*, germinated at a cumulative rate of 34 and 48 percent respectively by early spring the following year.

11. Survival of *M. spicatum* seedlings *in situ* is likely restricted by factors such as competition and shading from other aquatic plants, water depth and exposure to wave action. Under suitable habitat conditions, it appears likely that *M. spicatum* seeds could germinate and grow to mature size. Propagation of *M. spicatum* by sexual reproduction is considered insignificant in lakes already infested with this nuisance aquatic plant. However, reproduction through seeds may be an advantage in the colonization of new habitats downstream, or through dispersal of seeds by waterfowl to remote water bodies.

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POOR

QUALITY

ORIGINAL